Integration of Ki-67 index into AJCC 2018 staging provides additional prognostic information in breast tumours candidate for genomic profiling

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Running title: Ki67 proliferation index enriches 2018 AJCC

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ABSTRACT

Background

The 8th edition of the American Joint Committee on Cancer (AJCC) staging system (2018) for breast cancer (BC) introduced the prognostic stage. Moreover, multigene assessment has been indicated to tailor staging in T1/T2/N0, ER-positive/HER2-negative BC. However, many National Health Systems do not provide reimbursement for routine testing. The aim of this study was to assess whether Ki67 proliferation index is prognostically relevant for patients candidate for molecular testing.

Methods

A retrospective series of 686 ER+/HER2- BC were reclassified using AJCC 2018, and in the group of 521 patients for which AJCC 2018 recommends molecular evaluation, we assessed the prognostic efficacy of a prognostic stage enriched by Ki67 (Ki67-PS), considering Ki67<20% an alternative to Recurrence Score<11 provided by Oncotype DX.

Results

We found that a group of BCs (35.6%, 58/163) assigned to IB by prognostic score, were downstaged to IA with Ki67-PS. The outcome of these 58 cases overlapped with that of lesions classified as stage IA using prognostic stage, showing a significantly better prognosis compared to IB tumors (HR = 2.79, p = 0.003).

Conclusions

These data suggest that Ki67 may be a reliable marker to enrich the 2018 AJCC prognostic score in BC patients candidate for genomic profiling.

Background

Breast cancer (BC) is the most common cancer in women. The clinical approach to this disease varied over the years from radical surgery and aggressive oncological therapy, to the minimal patient-tailored effective treatment.[1,2]

Recently, several studies demonstrated that the biological phenotype of the tumor may be a superior prognostic variable than lymph node staging.[3] In particular, Mittendorf et al. described that among T1 BC patients, estrogen receptor (ER) status and histological grade are better predictors of survival than presence of small-volume nodal metastases.

Accordingly, the 8th edition of the American Joint Committee on Cancer (AJCC) staging system, published in 2018, proposed the use of a dual approach based on the traditional anatomic stage (AS) (*i.e.* tumor size, lymph node status), which remains unchanged from the 7th AJCC edition and the novel prognostic stage (PS). This latter takes into account biological information, such as ER, Progesterone Receptor (PR), HER2 status and histological grade and integrates them with AS.

To optimize patient care and in particular to allow appropriate treatment de-escalation, AJCC 2018 recommends molecular profiling in T1/T2 tumors without lymph nodes metastases and ER-positive/HER2-negative status. Specifically, four tools have been recommended: Oncotype DX® (level of evidence, I), Mammaprint®, Endopredict® and Breast Cancer Index® (level of evidence, II). In particular, the AJCC suggested that independently from anatomic stage, ER-positive/HER2-negative tumor should be reclassified as stage IA in case of recurrence score (RS) <11 by Oncotype DX®.

To date, in many European countries, including Italy, none of these molecular tests is reimbursed by the National Health System hampering the prompt translation of AJCC 2018 recommendations into the routine clinical practice. In addition, even if approved, these tests could hamper the budget sustainability of pathology laboratories.

The proliferation index, assessed using Ki67, is considered an important prognostic biomarker in BC.[4] Ki67 is typically useful in ER-positive/HER2-negative BC, to discriminate, together with PR, luminal A from luminal B cases, as recommended by St. Gallen guidelines.[5] Determination of Ki67 by immunohistochemistry (IHC) is routinely used to integrate the histology report and to add prognostic information, despite some criticism regarding its reproducibility[6] and different cut off values proposed in literature.[5,7,8]

Since most of the genes assessed by the previously listed molecular assays are related to cell proliferation, we hypothesized that a proliferative marker like Ki67 could partly substitute information obtained by genomic profiling.

The aim of the present study was to evaluate the efficacy of a Ki67-integrated AJCC 2018 prognostic stage (Ki67-PS) for prognostic assessment of patients candidate for molecular assays. In particular, we firstly reclassified a retrospective series of ER+/HER2- BC using both AJCC anatomic and prognostic stages. Then, in the subgroup of patients candidate for multigene panel evaluation according to AJCC, we tested the prognostic efficacy and reliability of a Ki67-integrated PS (Ki67-PS).

Methods

Case series

We retrospectively evaluated 686 ER+/HER2– BC patients who underwent conservative surgery at the Breast Unit of “Città della Salute e della Scienza” University Hospital (Turin, Italy) from April 1998 to December 2012. Data concerning tumor diameter, lymph node involvement, tumor grade, histological type, ER, PR, HER2, and Ki67 expression levels were obtained from the pathological reports. In addition, type of therapy and follow up status were collected from clinical reports. All the cases were anonymously recorded into a dedicated database, and data were accessed anonymously. The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and within the guidelines and regulations defined by

the Research Ethics Committee for human Biospecimen Utilization (Department of Medical Sciences – ChBU) of the University of Turin. Considering the retrospective nature of this research protocol, which involved only already existing medical data that were previously anonymized with no impact on patient care, no specific written informed consent was required by the Committee.

Immunohistochemistry

Tissue sections were routinely immunostained using an automated slide processing platform (Ventana BenchMark AutoStainer, Ventana Medical Systems, Tucson, AZ, USA) with the following primary antibodies: prediluted anti-ER rabbit monoclonal antibody (SP1, Ventana Medical Systems); prediluted anti-PgR rabbit monoclonal antibody (1E2, Ventana Medical Systems) and anti-Ki67 mouse monoclonal antibody (MIB1, diluted 1:50, Dako). Evaluation of HER2 expression was performed by an anti-HER2 polyclonal antibody (A0485, diluted 1:800, Dako). Fluorescence in situ hybridization (FISH) was performed to define HER2 status in IHC equivocal cases (score 2+).[9] Positive and negative controls were included for each immunohistochemical run.

Pathological evaluation

Tumor size was dichotomized at 15 mm, as suggested by previous studies.[10,11]

Cut-off for ER and PR positivity was determined at <1%, according to the Consensus of St. Gallen 2011[12] HER2 was evaluated as recommended by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP).[13] Ki67 proliferation index was assessed on surgical specimens and a minimum of 1000 cells were evaluated.[4] The surrogate of molecular subtypes obtained from ER, PR and HER2 IHC expression is summarized in Supplementary Table 1. Luminal subtypes were defined according to St. Gallen proposal[5] using a Ki67 cut-off value of 20% in line with previously published studies.[7,14]

Anatomic and prognostic staging

All cases (n=686) were firstly staged using anatomic and prognostic stages, then BC in which further molecular testing (T1/T2, N0, M0) would be recommended according to AJCC 2018 were selected (n=521).[15] We hypothesized that the expression of Ki67 may provide prognostic information related to those obtained by Oncotype DX. Thus, in analogy to Oncotype DX® RS <11, we selected a value of Ki67 <20% to identify tumors staged IIA and IB which could be reclassified as IA. In case of Ki67 values $\geq$20%, as for RS $\geq$11 the PS was not modified.

Statistical analysis

Categorical data were described as counts and percentages. Disease Free Interval (DFI) was determined from the date of diagnosis to the date of first recurrence (either locoregional recurrence or distant metastasis) or, if no recurrence occurred, analysis was censored at time of last follow up. DFI was estimated with the Kaplan–Meier analysis. The Cox model was used to assess the prognostic value of a series of patient and tumor characteristics. Hazard ratios (HRs) and 95% confidence intervals (CIs) were also calculated. The proportional hazard assumption (Schoenfeld residuals) was always satisfied. The performance of the AJCC 2018 was informally compared through the Harrell C or the Somer D discrimination statistics in which the higher value was representative of better system performance. The Akaike information criterion was also computed, a lower value indicating the better performance of the model. Data were analyzed with Stata (version 15; Stata Corporation, College Station, TX, US). Concordance among different classification systems were performed using K Cohen. A two-sided *P* value of less than .05 was considered statistically significant. All statistical tests were two-sided.

Results

Clinico-pathological characteristics

Clinical and pathological information of 686 patients are reported in Supplementary Table 2. Briefly, 59.5% of the tumors had a diameter <15 mm and 85% were classified as pT1; of these 42.1% were well differentiated (G1) and 11.4% were poorly differentiated (G3). Lymph nodes resulted free of metastases in 76.1% of cases. The proliferation rate was low (Ki67 <20%) in 74.1% of cases. Most of tumors expressed PR and 59.3% were classified as Luminal A. All patients were treated by conservative surgery followed by radiotherapy. Hormonal therapy was administrated to 95.2% of patients, while 23% received chemotherapy. Distant or local relapse was observed in 58 patients (8.4%) and 21 died of BC (3.1%).

Classification using AJCC 2018

Patients were staged according to the AJCC 2018 anatomic staging (Fig. 1 - AS). According to this system, 468 (68.2%), 28 (4.1%), 132 (19.2%) and 39 (5.7%) of tumors were staged as IA, IB, IIA and IIB respectively, whereas 19 (2.7%) were in stage III (Supplementary Table 3).

Then, we re-staged the tumors using AJCC 2018 prognostic stage (Fig. 1 - PS). Applying this staging system, the majority of tumors were still classified as IA (63.7%); however, the prognostic stage reassigned to IA and IB stage the majority of patients previously classified as IB or IIA by anatomic stage (Supplementary Table 3).

Conversely, 57 cases changed from IA by anatomic stage to IB (51) and IIA (6) according to prognostic stage. Only 15 out of 39 cases staged as IIB by anatomic stage were confirmed by prognostic stage, while 14 cases were upstaged into IIIA, 2 were assigned to IIIB and 8 were down staged to IB (Supplementary Table 3).

Supplementary Table 4 summarized the results obtained by anatomic and prognostic stages, grouping stage I-II-III patients. Using the new prognostic classification proposed by AJCC the majority of patients of our series were shifted in stage I [K=0.38, IC95% (0.33-0.41)]. In particular, using the anatomic stage 5.6% of cases were stage IB, the rate increased to 27.2% using the prognostic stage.

Ki67-integrated Prognostic Stage (Ki67-PS)

We selected 521 patients with BC staged as T1/T2N0M0 that were potential candidates for molecular assessment following AJCC 2018. Differences between AJCC 2018 anatomic and prognostic staging are summarized in Supplementary Table 5. In this subgroup, Ki67 proliferation index was used to integrate the prognostic stage with additional information regarding biological aggressiveness (Ki67-PS) (Fig. 1 - Ki67-PS).

Clinical and pathological information of this patient group are reported in Table 1. As shown in Table 2, 411 patients remained assigned to IA stage using both prognostic stage and Ki67-PS, while 58 out of 89 (65,2%) and 3 out of 19 (15,8%) BCs previously classified as IB and IIA respectively were downstaged to IA, using Ki67-PS. In terms of absolute differences 61/521 (approximately 12%) patients were differently classified.

Table 3 summarizes the results obtained by the three different staging systems, grouping stage I-II-III patients. Prognostic staging (95.9%) and Ki67-PS (96.5%) moved to stage I the majority of BCs. In general, we observed an overlap between prognostic stage and Ki67-PS, although stage IA counted more cases (411 vs 472) according to Ki67-PS.

Outcome analysis according to different staging systems

To understand which staging system could be more accurate to predict the prognosis in ER+ BC patients, we used Kaplan Meier analysis (Fig. 2 A-C). Only prognostic stage and Ki67-PS clearly distinguished stage I from stage II and III (Log-rank test $p<0.001$) (Fig. 2B and 2C, respectively). In addition, a significant difference of DFI among stages (I-II-III) was observed at univariate analyses regardless of the staging system used (Table 4).

Based on prognostic stage, DFI was significantly different in stage IA and IB (Log-rank test $p<0.001$) (Fig. 2D). In particular, the 58 cases that were downstaged from IB to IA using Ki67-PS

showed a favorable outcome, similar to those classified as stage IA (p=0.307). (Fig. 2D, Table 4) and a better prognosis compared to IB lesions (HR=2.79, p=0.003).

Discussion

In the present study a retrospective series of ER+/HER2- BC with long follow up was reclassified using both 8th edition AJCC anatomical and prognostic stages. The results obtained confirm that integration of tumor load (size and presence of node involvement) with tumor type (grade and prognostic factors) leads to an increased number of patients classified as Stage I, as previously reported.[16,17] Furthermore, in line with other studies,[18,19] we found that stage I according to prognostic stage clearly identifies a group of patients with a more favorable outcome, distinguishing them from other patients with lesions classified as stage II or III and providing more accurate prognostic information compared with anatomic stage.

To furtherly improve patient care and avoid unnecessary treatments, AJCC 2018 recommends the use of multigene profiling in the subset of T1/T2-N0, HER2-negative luminal BCs.

However, in many countries, including Italy, the National Health System does not reimburse these tests, hampering the prompt translation of AJCC 2018 recommendations into the routine clinical practice.

In absence of molecular assays, Ki67 is to date the only recommended marker, together with PR, that can help oncologists to differentiate luminal A from luminal B surrogate categories.[8]

In the present study, we created a prognostic stage integrated with Ki67 (Ki67-PS), hypothesizing that expression of Ki67 may stratify patients similarly to Oncotype DX®. Actually, Oncotype DX® is based, among others, on the expression of 5 genes related to proliferation

(namely MKI67, STK15, Survivin, CCNB1, and MYBL2), and the association between both, RS and single gene expression, with the Ki67 IHC levels has previously been addressed.[20–23]

Since use of Oncotype DX® in routine practice requires important financial resources and its cost-effectiveness has been questioned in the literature,[24,25] especially for low risk BC patients, Ki67-PS can possibly provide additional information with an inferior burden on National Health System budget.

Several works reported a poor reproducibility of Ki67 assessment due to the use of different clones (e.g. MIB-1, MM1, NCL-Ki-67p)[26] and different pre-analytic procedures, as well as discordant diagnostic evaluation even in case of dedicated breast pathologists.[27] To overcome this problem, in Italy, breast pathologists and breast pathological labs perform routinely local, regional and national quality controls, to standardize pre-analytical and analytical assessment of this marker, according to recommendation by the St Gallen consensus conference.[5] In addition, our and other groups demonstrated that 20% is an optimal cut off of Ki67 to stratify patients with luminal BCs.[14,28,29] Thus, we hypothesized that tumors showing Ki67 <20% may be classified as stage IA, similarly to those with RS <11.

In the present study, we showed that prognostic score clearly separates stage I tumors from the others. However, using the integrated Ki67-PS, 61/521 (12%) patients were downstaged from IB (58 patients) and from IIA (3 patients) to IA with an outcome comparable to those classified as stage IA defined by prognostic stage in terms of DFI. These data support Ki67 as a possible marker to identify the subgroup of patients with luminal BC with good prognosis in which treatment de-escalation could be considered.

The present study has some limitations that warrant consideration. Its retrospective nature limits the collection of follow up data. Due to the small number of patients that died of disease, we could not perform survival analyses. However, to the best of our knowledge, this is the first study that reports effective integration of the newly introduced AJCC 2018 prognostic staging system with Ki67 IHC evaluation.

In conclusion, our results confirmed that prognostic stage provides better prognostic information compared to anatomic stage in luminal BC patients. Moreover, the use of Ki67-integrated prognostic stage may be a reliable method to obtain additional prognostic data, enriching the 2018 AJCC system in BC patients candidate for genomic profiling.

Additional Information

Ethics approval and consent to participate: Ethical approval for this study was obtained from the Committee for human Biospecimen Utilization (Department of Medical Sciences – ChBU). Considering the retrospective nature of this research protocol, which involved only already existing medical data that were previously anonymized with no impact on patient care, no specific written informed consent was required by the Committee. The study was performed in accordance with the Declaration of Helsinki.

Consent for publication: Not applicable.

Data availability: The dataset analyzed during the current study is available from the corresponding author on reasonable request. Data generated during this study are included in this published article [and its supplementary information files].

Conflict of interest: The authors have declared no conflicts of interest.

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Authors' contributions: I.C. conceived and designed the study. S.O.A. and G.M. performed statistical analyses. I.C., E.V., L.B., J.M., and P.C. evaluated and interpreted obtained data. I.C., E.V., J.M., L.B., P.C. and A.S. wrote the original draft. All authors contributed to reviewing the manuscript, its organization and approved the submitted and final version.

REFERENCES

1. Criscitiello C, Curigliano G, Burstein HJ, Wong S, Esposito A, Viale G *et al.* Breast conservation following neoadjuvant therapy for breast cancer in the modern era: Are we losing the opportunity? *Eur J Surg Oncol* 2016, 42, 1780–1786.
2. Galimberti V, Cole BF, Viale G, Veronesi P, Vicini E, Intra M *et al.* Axillary dissection versus no axillary dissection in patients with breast cancer and sentinel-node micrometastases (IBCSG 23-01): 10-year follow-up of a randomised, controlled phase 3 trial. *Lancet Oncol* 2018, 19, 1385–1393.
3. Mittendorf EA, Ballman KV, McCall LM, Yi M, Sahin AA, Bedrosian I *et al.* Evaluation of the stage IB designation of the American Joint Committee on Cancer staging system in breast cancer. *J Clin Oncol* 2015, 33, 1119–1127.
4. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J *et al.* Assessment of Ki67 in Breast Cancer: Recommendations from the international Ki67 in breast cancer working Group. *J Natl Cancer Inst* 2011, 103, 1656–1664.
5. Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart MJ *et al.* Tailoring therapies-improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. *Ann Oncol* 2015, 26, 1533–1546.
6. Varga Z, Cassoly E, Li Q, Oehlschlegel C, Tapia C, Lehr HA *et al.* Standardization for Ki-67 assessment in moderately differentiated breast cancer. A retrospective analysis of the SAKK 28/12 study. *PLoS One* 2015, 10, 1–13.
7. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart MJ, Thürlimann B *et al.* Personalizing the treatment of women with early breast cancer: Highlights of the st gallen international expert consensus on the primary therapy of early breast Cancer 2013. *Ann Oncol* 2013, 24, 2206–2223.
8. Curigliano G, Burstein HJ, Winer EP, Gnant M, Dubsky P, Loibl S *et al.* De-escalating and

escalating treatments for early-stage breast cancer: The St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. *Ann Oncol* 2017, 28, 1700–1712.

9. Marchiò C, Lambros MB, Gugliotta P, Di Cantogno LV, Botta C, Pasini B *et al.* Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence in situ hybridization and microarray-based CGH analysis. *J Pathol* 2009, 219, 16–24.

10. Castellano I, Chiusa L, Vandone AM, Beatrice S, Goia M, Donadio M *et al.* A simple and reproducible prognostic index in luminal ER-positive breast cancers. *Ann Oncol* 2013, 24, 2292–2297.

11. Duffy SW, Tabar L, Vitak B, Warwick J. Tumor size and breast cancer detection: What might be the effect of a less sensitive screening tool than mammography? *Breast J* 2006, 12, S92-S95.

12. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn H-JJ *et al.* Strategies for subtypes-dealing with the diversity of breast cancer: Highlights of the St Gallen international expert consensus on the primary therapy of early breast cancer 2011. *Ann Oncol* 2011, 22, 1736–1747.

13. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH *et al.* Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *J Clin Oncol* 2013, 31, 3997–4013.

14. Bustreo S, Osella-Abate S, Cassoni P, Donadio M, Airoldi M, Pedani F *et al.* Optimal Ki67 cut-off for luminal breast cancer prognostic evaluation: a large case series study with a long-term follow-up. *Breast Cancer Res Treat* 2016, 157, 363–371.

15. Amin MB, Edge S, Greene F, Byrd DR, Brookland RK, Washington MK *et al* (eds). *AJCC cancer staging manual*, 8th edn. Springer International Publishing: New York, 2017.

16. Ibis K, Ozkurt S, Kucucuk S, Yavuz E, Saip P. Comparison of Pathological Prognostic Stage

and Anatomic Stage Groups According to the Updated Version of the American Joint Committee on Cancer (AJCC) Breast Cancer Staging 8th Edition. *Med Sci Monit* 2018, 24, 3637–3643.

17. Jang N, Choi JE, Kang SH, Bae YK. Validation of the pathological prognostic staging system proposed in the revised eighth edition of the AJCC staging manual in different molecular subtypes of breast cancer. *Virchows Arch* 2019, 474, 193–200.

18. Ye J, Wang W, Xu L, Duan X, Cheng Y, Xin L *et al.* A retrospective prognostic evaluation analysis using the 8th edition of American Joint Committee on Cancer (AJCC) cancer staging system for luminal A breast cancer. *Chinese J Cancer Res* 2017, 29, 351–360.

19. Xu L, Li J-H, Ye J-M, Duan X-N, Cheng Y-J, Xin L *et al.* A Retrospective Survival Analysis of Anatomic and Prognostic Stage Group Based on the American Joint Committee on Cancer 8th Edition Cancer Staging Manual in Luminal B Human Epidermal Growth Factor Receptor 2-negative Breast Cancer. *Chin Med J (Engl)* 2017, 130, 1945–1952.

20. Iwamoto T, Katagiri T, Niikura N, Miyoshi Y, Kochi M, Nogami T *et al.* Immunohistochemical Ki67 after short-term hormone therapy identifies low-risk breast cancers as reliably as genomic markers. *Oncotarget* 2017, 8, 26122–26128.

21. Thakur SS, Li H, Chan AMY, Tudor R, Bigras G, Morris D *et al.* The use of automated Ki67 analysis to predict Oncotype DX risk-of-recurrence categories in early-stage breast cancer. *PLoS One* 2018, 13, e0188983.

22. Xu C, Yamamoto-Ibusuki M, Yamamoto Y, Yamamoto S, Fujiwara S, Murakami K *et al.* High survivin mRNA expression is a predictor of poor prognosis in breast cancer: A comparative study at the mRNA and protein level. *Breast Cancer* 2014, 21, 482–490.

23. Thomas C, Robinson C, Dessauvagie B, Wood B, Sterrett G, Harvey J *et al.* Expression of proliferation genes in formalin-fixed paraffin-embedded (FFPE) tissue from breast carcinomas. Feasibility and relevance for a routine histopathology laboratory. *J Clin Pathol* 2017, 70, 25–32.

24. Wang S-Y, Chen T, Dang W, Mougalian SS, Evans SB, Gross CP. Incorporating Tumor Characteristics to Maximize 21-Gene Assay Utility: A Cost-Effectiveness Analysis. *J Natl Compr Cancer Netw* 2019, 17, 39–46.

25. Wang SY, Dang W, Richman I, Mougalian SS, Evans SB, Gross CP. Cost-Effectiveness analyses of the 21-Gene assay in breast cancer: Systematic review and critical appraisal. *J Clin Oncol* 2018, 36, 1619–1627.

26. Lindboe CF, Torp SH. Comparison of Ki-67 equivalent antibodies. *J Clin Pathol* 2002, 55, 467–471.

27. Polley M-YC, Leung SCY, McShane LM, Gao D, Hugh JC, Mastropasqua MG *et al.* An International Ki67 Reproducibility Study. *J Natl Cancer Inst* 2013, 105, 1897–1906.

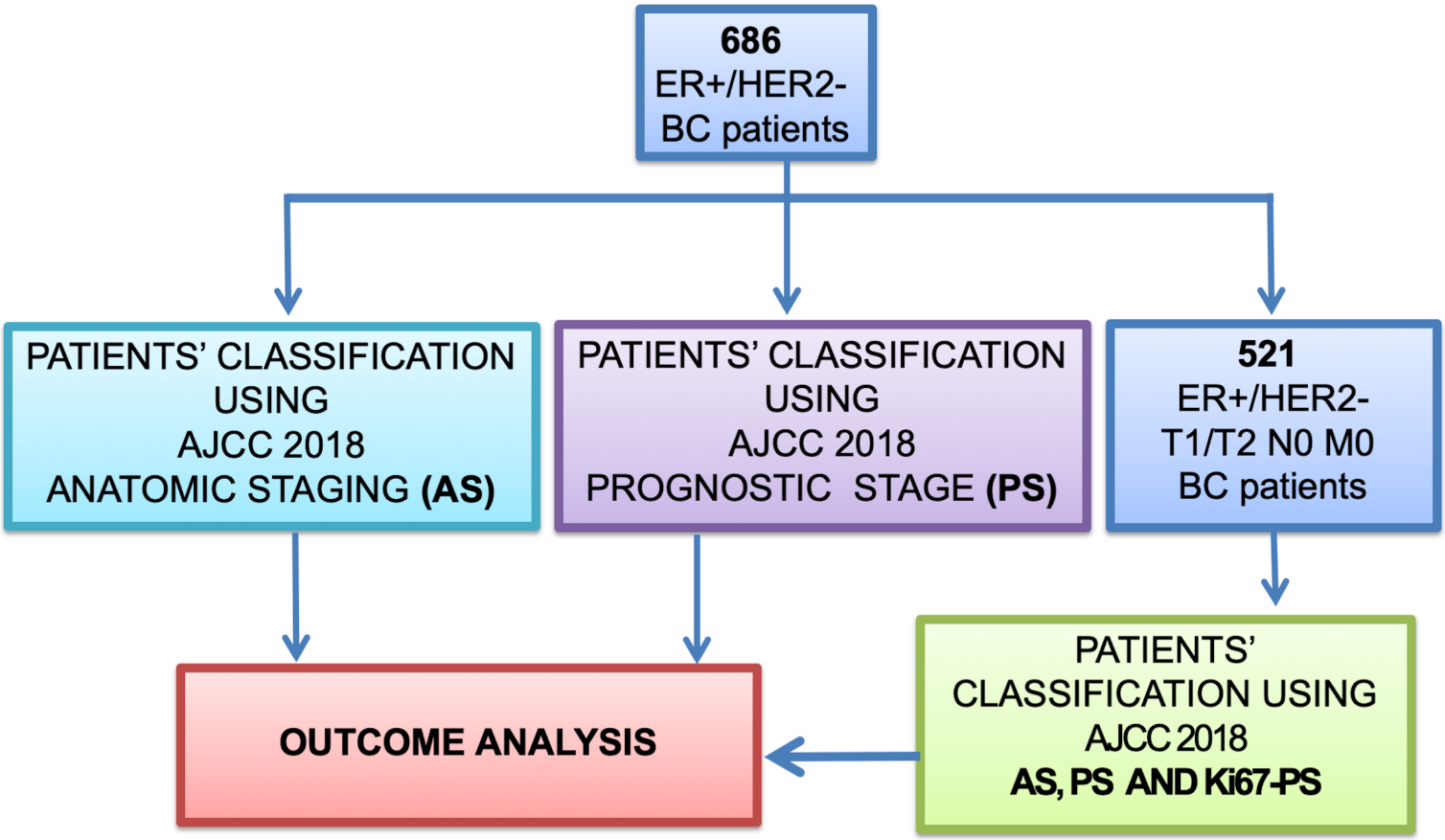
28. Tashima R, Nishimura R, Osako T, Nishiyama Y, Okumura Y, Nakano M *et al.* Evaluation of an optimal cut-off point for the Ki-67 index as a prognostic factor in primary breast cancer: A retrospective study. *PLoS One* 2015, 10, 1–10.

29. Chen Y-Y, Tseng L-M, Yang C-F, Lien P-J, Hsu C-Y. Adjust cut-off values of immunohistochemistry models to predict risk of distant recurrence in invasive breast carcinoma patients. *J Chinese Med Assoc* 2016, 79, 649–655.

Figure Legends

Fig. 1: Study flowchart.

Fig. 2: Disease Free Interval (DFI) of stage I-II-III assessed using AJCC 2018 anatomical stage (log-rank test p<0.001) (**A**), prognostic stage (log-rank test p<0.001) (**B**) and Ki67-PS (log-rank test p<0.001) (**C**) (Kaplan Meier analysis). DFI of stage IA and IB assessed using prognostic stage and of stage IA obtained from downstaging of IB using Ki67-integrated prognostic score (Ki67-PS) (log-rank test p<0.001) (Kaplan Meier analysis) (**D**).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OUTCOME ANALYSIS

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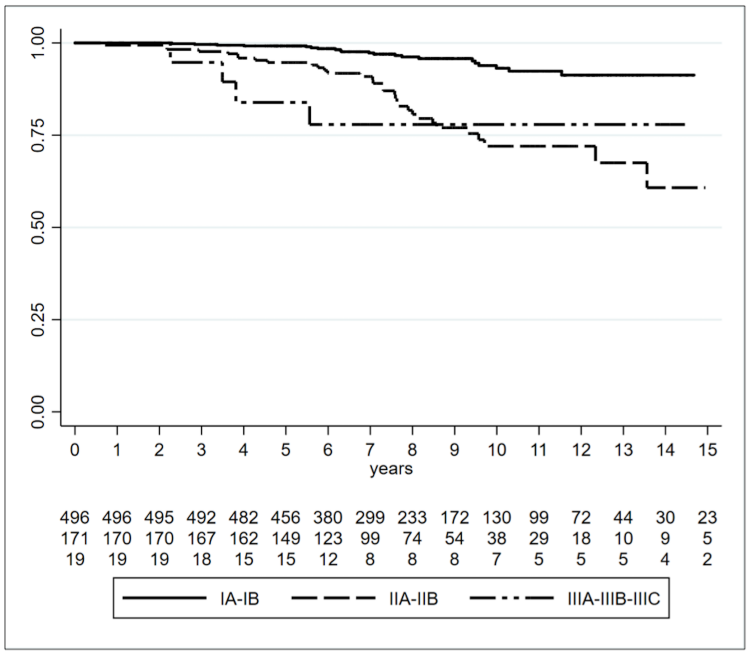
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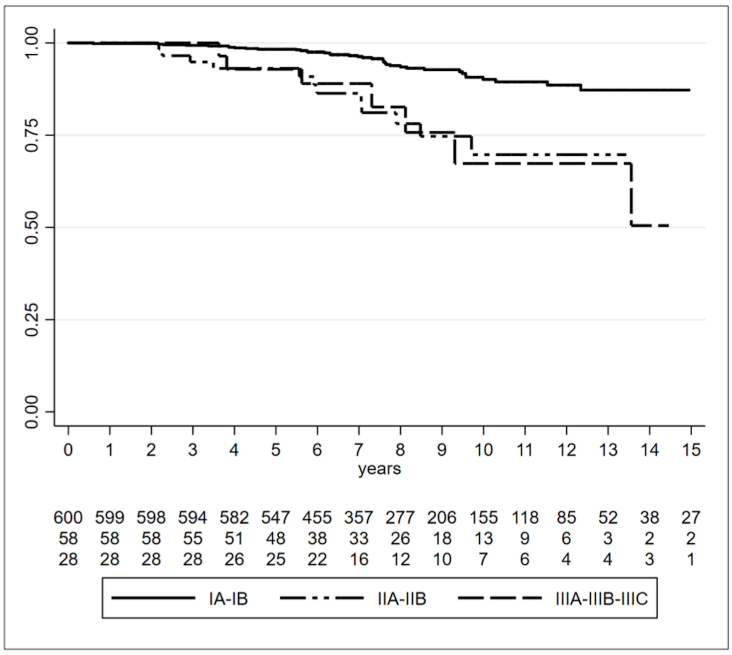
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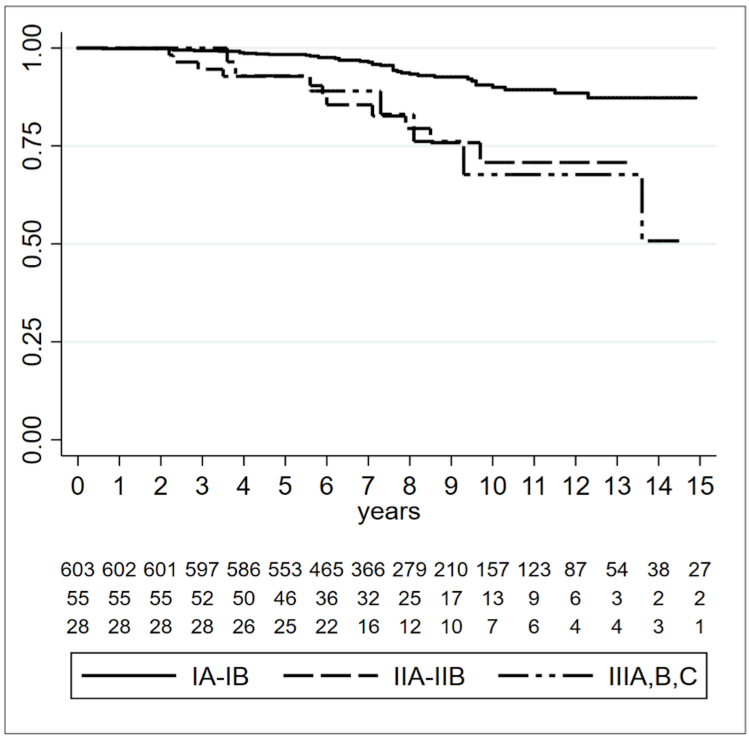
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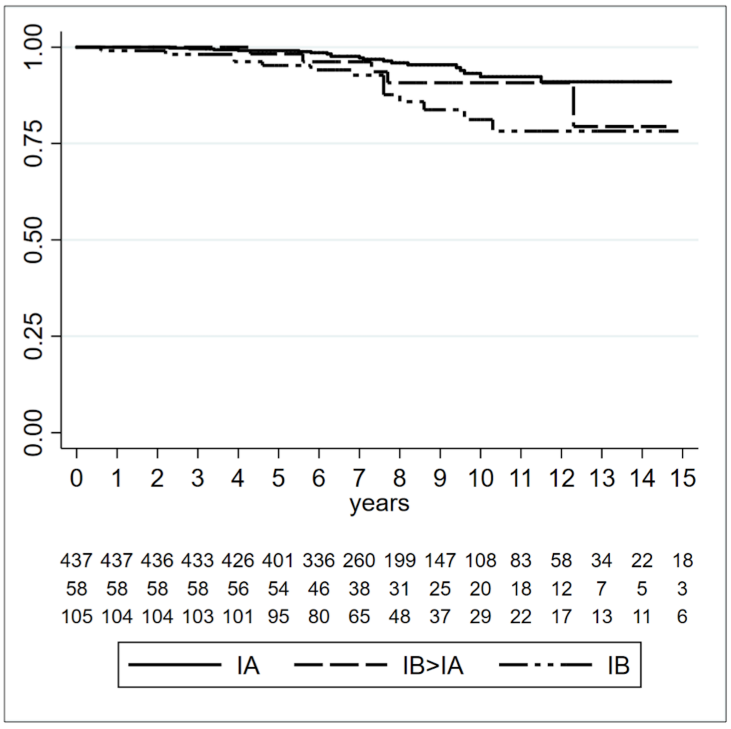
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IB

Table 1: Clinical and pathological characteristics of patients candidate for molecular profiling

	N. of patients 521	***%***
Diameter		
<15 mm	343	65,8
≥15 mm	178	34,2
pT		
1	468	89,8
2	53	10,2
Grade		
1	231	44,3
2	244	46,8
3	46	8,8
Ki67		
<20%	404	77,5
≥20%	117	22,5
PR*		
Negative	33	6,3
Positive	488	93,7
Subtype		
Luminal A	319	61,2
Luminal B	202	38,8
Chemotherapy		
No	468	89,8
Yes	53	10,2
Recurrences		
No	491	94,2
Yes	30	5,8

**(PR = Progesterone Receptor)*

Table 2: Classification of 521 BC patients according to Prognostic Stage 8th edition AJCC 2018 and Prognostic Stage modified using Ki67 (Ki67-PS)

		AJCC 2018 Prognostic Stage modified by Ki67 (Ki67-PS)					
		IA	IB	IIA	IIB	IIIA	Total
AJCC 2018 Prognostic Stage	IA	411	0	0	0	0	411
	IB	**58**	31	0	0	0	89
	IIA	**3**	0	16	0	0	19
	IIB	0	0	0	0	0	0
	IIIA	0	0	0	0	2	2
	IIIB	0	0	0	0	0	0
	IIIC	0	0	0	0	0	0
Total		472	31	16	0	2	521

Table 3: Classification of 521 BC patients following 8th edition AJCC 2018 (AS, PS and Ki67-PS)

AJCC 2018 ANATOMIC STAGE	**Stage I**		**Stage II**		**Stage III**
	468		53		0
	IA	IB	IIA	IIB	IIIA
	468	0	53	0	0
AJCC 2018 PROGNOSTIC STAGE	**Stage I**		**Stage II**		**Stage III**
	500		19		2
	IA	IB	IIA	IIB	IIIA
	411	89	19	0	2
AJCC 2018 PROGNOSTIC STAGE WITH Ki67	**Stage I**		**Stage II**		**Stage III**
	503		16		2
	IA	IB	IIA	IIB	IIIA
	472	31	16	0	2

Table 4: Univariate analyses on DFI across different staging systems proposed by 8th edition AJCC 2018 and using Ki67 integrated PS

System Classification		HR	CI	*p-value*
AJCC 2018 Anatomic Stage (AS) Harrel c test 0.6993 AIC 672.6299	I	1		
	II	4.54	2.63-7.82	<0.001
	III	4.62	1.58-13.48	0.005
AJCC 2018 Prognostic stage (PS) Harrel c test 0.6993 AIC 672.6299	I	1		
	II	3.44	1.80-6.57	<0.001
	III	3.87	1.73-8.66	0.005
AJCC 2018 PS integrated by Ki67 (Ki67-PS) Harrel c test 0.6094 AIC 674.1635	I	1		
	II	3.27	1.67-6.36	0.001
	III	3.79	1.70-8.47	0.001
AJCC 2018 PS and Ki67-PS Harrel c test 0.6265	IA	1		
	IB>IA	1.66	0.62-4.44	0.307
	IB	2.79	1.41-5.53	0.003